HISTAMINE, SEROTONIN AND MAST CELLS IN THE SKIN OF THE RAT DURING THE HAIR CYCLE*

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The probability that mast cells in general, and histamine in particular, may somehow influence the development of hair follicles has already been suggested on both theoretical and experimental grounds (1, 2, 6, 23, 24). The profound anatomical changes occurring in skin blood vessels during the hair cycle could, for instance, in part be related to the histamine, serotonin, and heparin contained within the mast cells (9, 10, 14, 17, 21).

The present report is an analysis of the quantitative behavior of the mast cell population of the skin and histamine and serotonin content during the various stages of rat's hair cycle.

MATERIALS AND METHODS

Three hundred and fifty Wistar S.M., 62 day old albino rats were used for studying 4 spontaneous and 3 induced hair cycles, each lasting approximately 31 days (Anagen: about 8 days; Catagen: 3-4 days; Telogen: about 10 days (5)).

Animals were killed on the 5th, 11th, 16th, 19th, 23rd, 28th day of the spontaneous cycle, and the 2nd, 7th, 13th, 18th, 21st, 25th, 30th day of each induced cycle.

The skin from the posterior-most part of the back was shaved, excised, and, after being thoroughly washed, freed from all visible hanging fat, was divided into many small samples as indicated in figure 1.

The skin specimens to be used for histochemical investigations were fixed 1 to 4 hours in 10% cold, neutral, unbuffered formalin, while those to be used for chemical and biological technics were dried in an oven for 90 minutes at 80°C.

A. The identification and the counting of mast cells:

(a) Mast cells were revealed in paraffin sections stained with toluidin blue (16); other sections were stained with hematoxylin and eosin.

(b) The density of mast cell population was calculated for every day in the skin with spontaneous cycles, and on a few specified days in that of artificially induced cycles, by counting all recognizable mast cells within 18 microscopic fields, amounting to a total of 1,051 mm.^{2} of skin surface. Counts were repeated on 5 different 10 μ thick serial sections, 250 μ apart over an area of 1 mm. (8).

B. Chemical determinations of total nitrogen and chemical-biological and pharmacological technics for histamine and serotonin:

(a) The total nitrogen content per gram of skin (dry weight) was obtained by a standard micro-Kjeldahl technic.

(b) Histamine was extracted and quantitatively and qualitatively evaluated, in 3 different samples, in a thermostatically controlled bath at 37°C on perfused and atropinized guinea-pig ileum (11).

(c) Serotonin was extracted and also evaluated in 3 different samples, in a thermostatic bath at 20°C on perfused and atropinized rat colon (25).

(d) The pharmacological identity of histamine (1 γ of histamine base = 1.66 γ histamine dihydrochloride) was checked and confirmed by adding to the thermostatic bath an antihistamine (Trimeton); to that of serotonin were added the required amounts of LSD (diethylamide of lysergic acid), dihydroergotamine, or large amount of serotonin itself (25).

C. Statistical evaluation of results:

A statistical evaluation was made by adding each of the 4 groups of results (mast cell counts, total nitrogen, histamine and serotonin determinations) in each rat to those of the rats killed the same day, calculating their average values, their standard error (S.E.), and studying the significance of the differences among the average of the various days according to the formula (12):

$$t = \frac{\bar{x} - \bar{y}}{\sqrt{\dfrac{\Sigma(\bar{x} - x)^2 + \Sigma(\bar{y} - y)^2}{N_x + N_y - 2}}} \cdot \sqrt{\frac{N_x N_y}{N_x + N_y}}$$

RESULTS

The density of mast cell population and the contents of total nitrogen, histamine, and serotonin revealed considerable quantitative changes in all hair cycles both spontaneous and artificially induced.

(a) Large numbers of various morphologic types of mast cells (2) are readily seen in histologic sections on the first days of spontaneous cycle. Their population radically decreases during late anagen, reaching the lowest density on the 19th day of the cycle in catagen, after which their number rises again in telogen (Figs. 2, 6).

These results are supported by the statistical

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Received for publication June 15, 1962.

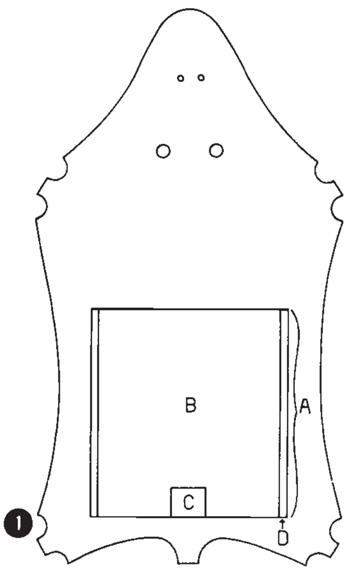


FIG. 1. Diagram of the epilated area "A"; of this area the "B" fraction was used for the determinations of histamine, serotonin, and total nitrogen; the "C" fraction was used for mast cells counts; the "D" fraction for routine hematoxylin and eosin stains.

evaluation of the data (Table 1). The mast cell population during the spontaneous cycles was compared with that in the artificially induced cycles. We think, therefore, that the curve in Figure 2 is probably valid for both cycles.

(b) During anagen the total skin nitrogen content (Figs. 3, 4) rises continuously reaching a peak between the 16th and the 18th day; thereafter it quickly diminishes throughout catagen, and returns almost to the initial values in telogen. These changes are statistically significant; compare, for example, the differences in the average values (according to the type of cycle) of the 31st day vs. the 16th day, the 16th day vs. the 28th day, or the 2nd vs. the 21st and the 21st vs. the 30th day (Table 2).

(c) During anagen, the content of skin histamine (Figs. 4, 6) increases rapidly in the first week, remains practically unchanged through most of the second week and drops about 30% in the last days. This fall, although less rapid, continues in catagen and part of telogen. During the latter part of telogen there is a renewed increase in histamine content, but the amount does not reach the values obtained at the beginning of the cycle.

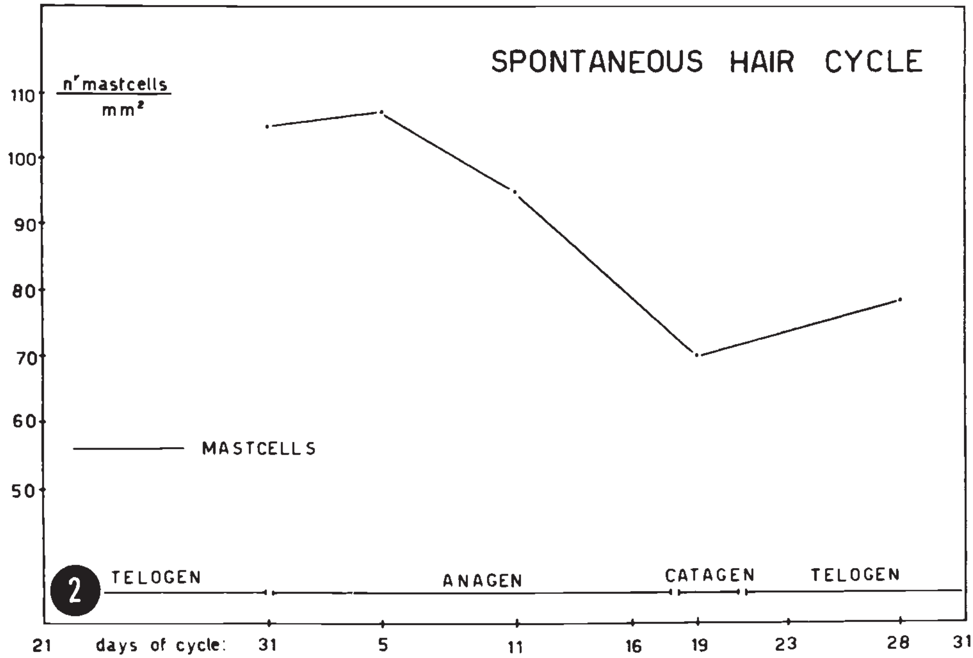
A statistical analysis of these fluctuations (Table 3) generally supports the above data, particularly in regard to the significance of the drop obtained between the 11th and 16th day and the 13th and the 18th day. Since the curve for histamine/total nitrogen ratio (Fig. 5) closely resembles the curve for histamine alone, the rise of histamine in the first week of anagen seems to be independent of the simultaneous increase in nitrogenous material. The amounts of histamine extracted from the skin of both spontaneous and artificial cycles, in general, are very similar (Table 2).

(d) During anagen, in induced hair cycles, the serotonin content (Figs. 3, 6) progressively decreases from 1.5 γ to 0.7 γ/gdw; in contrast, during catagen and telogen skin serotonin increases again reaching values of 1.2 γ, *i.e.*, lower at the beginning than at the end of the cycle. Similar changes may also occur in the spontaneous cycle. These results are confirmed by statistical analysis (Table 4). As with histamine, there was a basic agreement between the amounts of the amine extracted from the induced and the spontaneous hair cycle (Table 4).

CONCLUSIONS AND COMMENTS

The changes described in the mast cell population and histamine and serotonin contents of the skin seem to be directly related.

An analysis of these changes suggests that: (a) the increase in total nitrogen is possibly related to the development of hair follicles and the general thickening of skin during anagen; its subsequent decrease parallels the dissipation of hair follicles and thinning of the skin in catagen and telogen (5, 7, 15, 18); (b) the changes in histamine content are largely independent of the total nitrogen fluctuations, being partly bound to the number of mast cells residing in the deeper dermis (21, 22) (Fig. 6). The presence of some histamine from pooled blood in the skin or epidermal histamine by no means should be excluded from these data (13, 20). (c) The nearly equal percentages of decrease and increase in serotonin values parallel the increase and decrease in total nitrogen values; this may indicate a "dilution" of the amine caused by the changes in volume of the skin during hair cycles (Fig. 3). The resemblance of the serotonin curve to the mast cell curve suggests, however, that a fraction



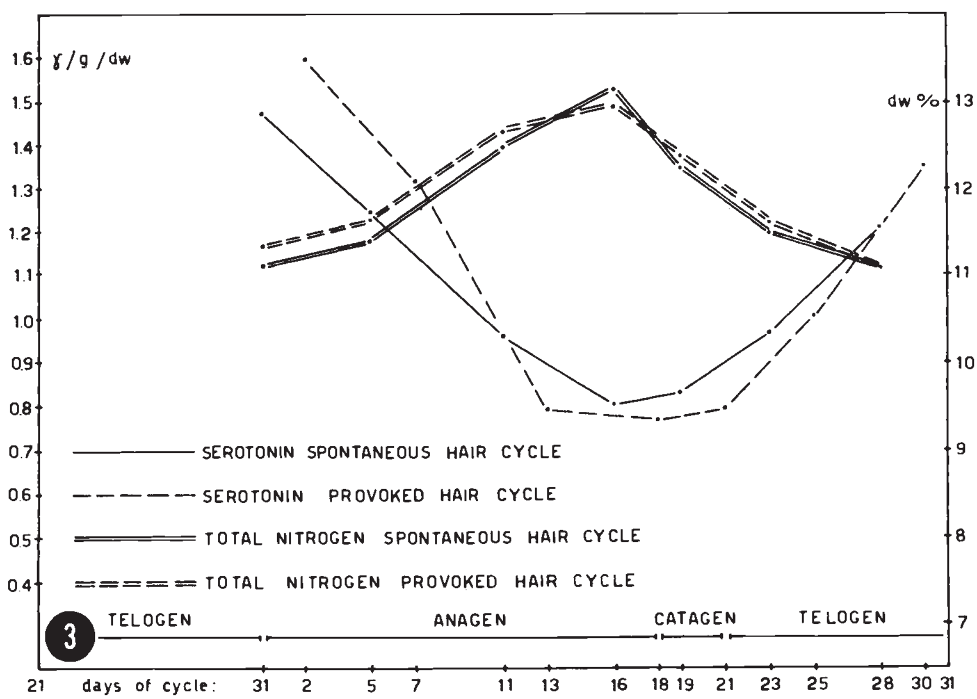


Fig. 2. The quantitative behaviour of mast cell population during spontaneous hair cycles.

Fig. 3. Quantitative changes of total nitrogen and serotonin during both spontaneous and provoked hair cycles.

TABLE 1

Mast cells population (spontaneous hair-cycle)

Hair-Cycle Phases	Experimental days	Mean Values/mm²	SEM	Significance of Differences		
				Compared days	"t"	%
Anagen	31	106.2	20.1	31–5	0.113	>90
	5	106.8	16.5	5–11	2.643	ca. 1.2
	11	96.6	9.8	11–19	7.386	<0.1
Catagen	19	73.3	12.3	19–28	2.595	ca. 1.3
Telogen	28	81.7	10.5	—	—	—

TABLE 2

Total nitrogen skin content

Spontaneous Hair-Cycle						Provoked Hair-Cycle						
Experimental days	Mean values % d.w.	SEM	Significance of differences			Hair cycle phases	Experimental days	Mean values % d.w.	SEM	Significance of differences		
			Compared days	"t"	%					Compared days	"t"	%
31	10.91	1.22	31–5	0.43	ca. 65	Anagen	2	12.53	0.45	2–7	2.73	ca. 1.4
5	11.20	1.02	5–11	2.04	ca. 7		7	12.77	0.24	7–13	1.85	ca. 8.2
11	12.19	0.60	11–16	1.72	ca. 12		13	13.21	0.67	13–18	0.28	ca. 78
16	12.84	0.64	16–19	2.00	ca. 7.9		18	13.28	0.52	18–21	2.28	ca. 3.5
19	12.01	0.70	19–23	1.04	ca. 32	Catagen	21	13.85	0.68	21–25	1.27	ca. 3.5
23	11.22	1.70	23–28	0.28	ca. 79	Telogen	25	13.25	1.46	25–30	1.50	ca. 15
28	10.97	1.46	31–16	3.16	ca. 1		30	12.57	0.91	2–21	6.23	<0.1
—	—	—	16–28	2.64	ca. 1.3		—	—	—	21–30	5.70	<0.1

of 5HTA could have originated from those cells (Fig. 6) (14).

Thus, the content of histamine and mast cells is apparently influenced by the structural changes (as revealed by total nitrogen fluctuations) in the skin during the stages of hair growth. The increase in mast cells and in histamine content occur while the skin is still relatively unaltered, between the end of telogen and the beginning of a new anagen phase. The question then arises if in rats a sudden liberation of histamine from mast cells may help initiate new hair follicles by the dilatation and enhanced permeability of the dermal vascular network.

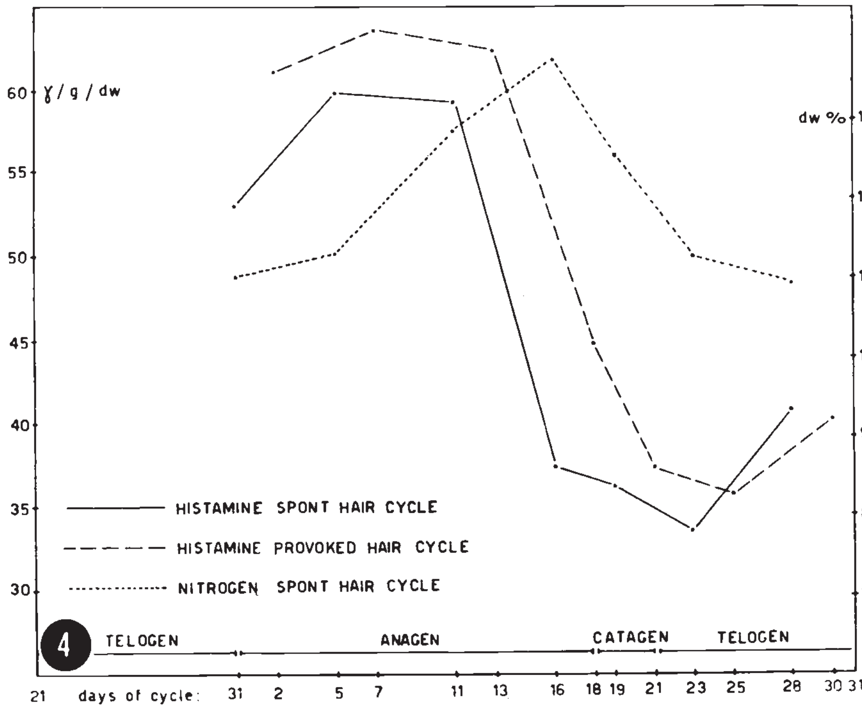
The following facts lend support to this hypothesis:

(1) Normal and alopecic scalps are equally richly supplied with variable amounts of mast cells (2).

(2) Animals treated with histamine show a faster rate of hair growth (6, 19, 24).

(3) The high histamine values we observed in the first 11 to 13 days of anagen in the rat are accompanied by a general increase in the number and diameter of all visible dermal blood vessels. When the values fall, 5 to 7 days later, there is a general collapse and atrophy of the vascular network (9).

(4) The high histamine values in the first 11 to 13 days of anagen also coincide with a high skin water content; the decrease of these values conversely coincides with a pronounced skin-



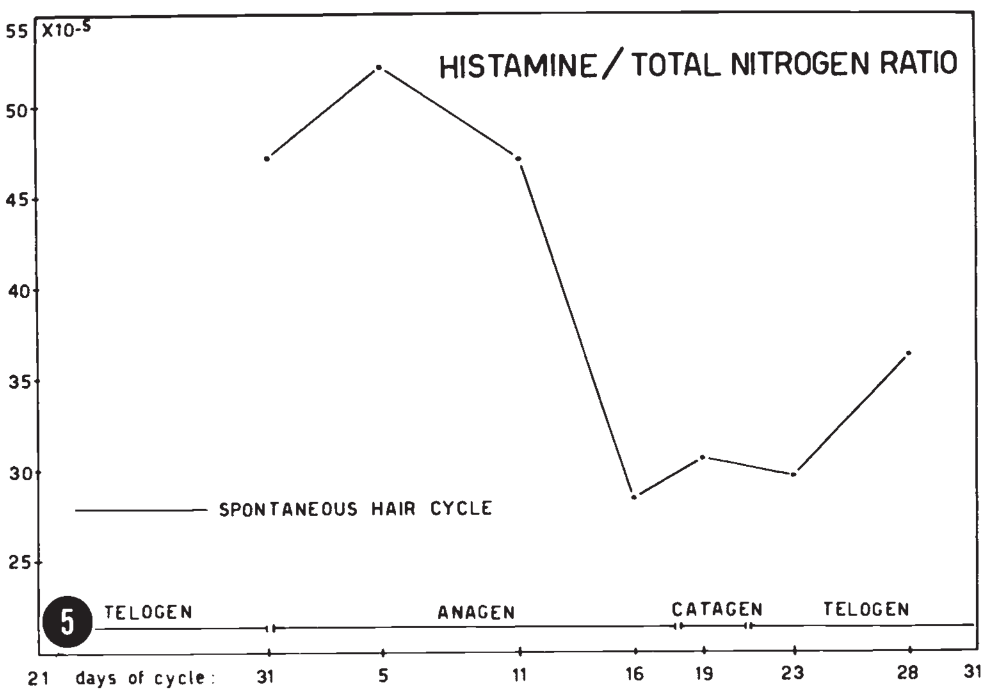


FIG. 4. Quantitative changes of histamine in both spontaneous and induced hair cycles. A curve showing the quantitative behaviour of nitrogen during a spontaneous cycle is superimposed to that of the histamine fluctuations during the same cycle.

FIG. 5. The behaviour of histamine-total nitrogen ratio during spontaneous hair growth cycles.

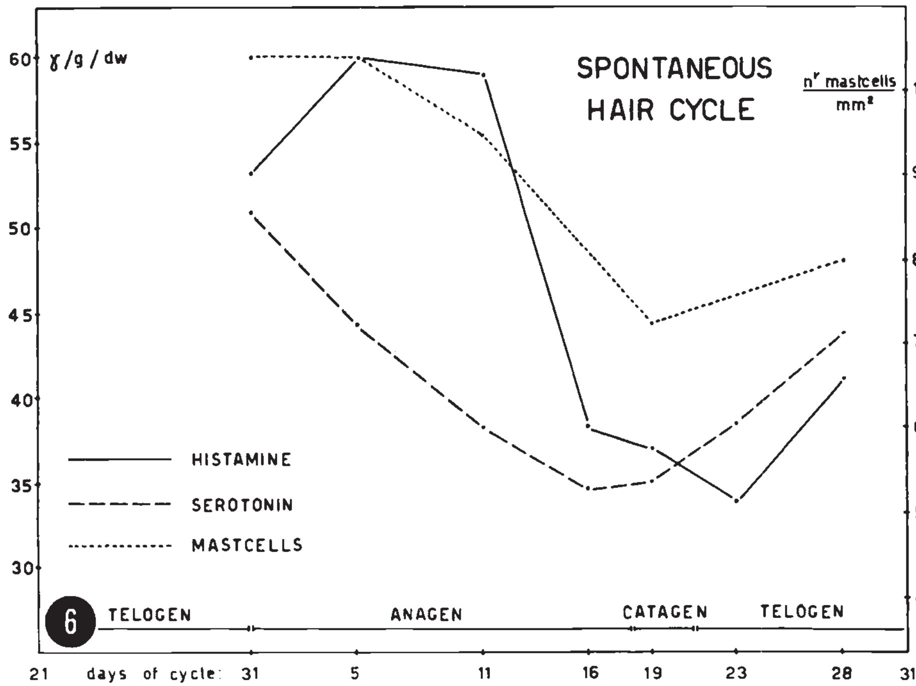


FIG. 6. A simultaneous comparison of the curves of mast cell population histamine and serotonin behaviour during the same spontaneous hair cycle.

TABLE 3

Histamine skin content

Spontaneous Hair-Cycle							Provoked Hair-Cycle					
Experimental days	Mean values γ/g. d.w.	SEM	Significance of differences: Compared days	"t"	%	Hair cycle phases	Experimental days	Mean values γ/g. d.w.	SEM	Significance of differences: Compared days	"t"	%
31	53.34	2.39	31–5	3.75	ca. 0.1	Anagen	2	61.36	3.14	2–7	2.67	ca. 1.9
5	60.35	5.40	5–11	0.27	ca. 79		7	64.03	1.64	7–13	1.47	ca. 15
11	59.70	5.04	11–16	13.19	0.1		13	63.20	1.84	13–18	6.15	0.1
16	37.82	1.42	16–19	0.55	ca. 59		18	45.67	8.08	18–21	2.90	ca. 1
19	36.70	5.95	19–23	1.50	ca. 16	Catagen	21	37.77	2.97	21–25	1.50	ca. 15
23	33.32	3.88	23–28	4.72	0.1	Telogen	25	35.76	3.00	25–30	3.78	ca. 0.1
28	41.00	3.36	16–23	3.43	ca. 0.1		30	40.63	2.73	18–25	3.62	ca. 0.1

water loss (3); in both cases however, simultaneous fluctuations of the very hydrophilic "ground substance" must be considered (4).

On the basis of our results, we think that histamine may in part be responsible for "triggering" the mechanism of the hair growth cycle in the rat. This conclusion, if correct, implies a relative lack of parallelism between the chronologic sequences of both biochemical and morphologic stages of hair cycles. This would be shown, for instance, by the decrease in histamine about 7 days earlier than the corresponding catagen vascular changes (9).

TABLE 4

Serotonin skin content

Spontaneous Hair-Cycle							Provoked Hair-Cycle					
Experimental days	Mean values γ/g. d.w.	SEM	Significance of differences: Compared days	"t"	%	Hair cycle phases	Experimental days	Mean values γ/g. d.w.	SEM	Significance of differences: Compared days	"t"	%
31	1.43	0.05	31–5	5.29	<0.1	Anagen	2	1.54	0.11	2–7	6.64	<0.1
5	1.19	0.09	5–11	22.4	<0.1		7	1.27	0.05	7–13	20.9	<0.1
11	0.90	0.04	11–16	6.69	<0.1		13	0.77	0.04	13–18	1.42	ca. 17
16	0.79	0.03	16–19	1.47	ca. 15		18	0.74	0.04	18–21	2.47	ca. 1.5
19	0.81	0.027	19–23	10.7	<0.1	Catagen	21	0.77	0.03	21–25	14.4	<0.1
23	0.94	0.027	23–28	9.35	<0.1	Telogen	25	0.98	0.02	25–30	9.24	<0.1
28	1.16	0.06	—	—	—		30	1.28	0.09	—	—	—

SUMMARY

During the three phases of the hair growth cycle in the rat, statistically significant fluctuations of skin mast cell population, histamine and serotonin content occur.

The mast cell population decreases sharply from the first week of anagen to catagen and rise in telogen. Histamine levels are considerably increased in the first week of anagen, and drop by about 30% in the following days until telogen when they increase again. Serotonin levels steadily diminish throughout anagen and rise through both catagen and telogen.

On the basis of these results and since the high histamine values of the first week of anagen are accompanied by an increase in the water content and vascularization of the skin, it seems possible that histamine, perhaps also liberated from the mast cells, may in part be responsible for "triggering" the mechanism of the hair-growth cycle in the rat.

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ANNOUNCEMENT

THIRD ANNUAL HERMAN BEERMAN LECTURE

TRANSPLANTATION: PAST, PRESENT AND FUTURE

RUPERT E. BILLINGHAM, M.D.

The Wistar Institute, Philadelphia, Pa.

At the 24th Annual Meeting of The Society for Investigative Dermatology, in Atlantic City, N. J., June 17–20, 1963, Dr. Rupert E. Billingham of The Wistar Institute, Philadelphia, Pa., will deliver the Third Annual Herman Beerman Lecture. Dr. Billingham's topic will be, "Transplantation: Past, Present and Future," and the lecture will be given on Wednesday, June 19th, at 9:00 A.M., at the Shelburne Hotel, Atlantic City, N. J.